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COMPLETE SPECIFICATION.

Utilization of Molasses Spent Wash.

We, JOH. A. BENCKISER G. m. b. H. CHEMISCHE FABRIK, of Frankenthaler Strasse 3, Ludwigshafen a.Rh., Germany, a German Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention concerns a process for the utilization of molasses spent wash by treatment with micro-organisms, for the production of animal feeding stuffs or nutritional factors.

The expression "spent wash" is used herein to signify the residue from fermented wort after the desired fermentation product has been removed. Such usage is standard in connection with the manufacture of alcohol and bakers' yeast; for convenience the same expression is used herein to designate aqueous effluents from other fermentations, for example, the liquor left after recovery of citric acid from the respective fermented wort. The expression "fungal carpet" denotes a supernatant mycelium, such as is formed in the course of non-submerged fermentative production of citric acid.

It has previously been attempted to utilize molasses spent wash in various ways. Thus, distillers' spent wash has been used as nutrient medium for the cultivation of yeast. Reference may be made, for example, to Patent Specification No. 541,594. However, this procedure has the drawback of entailing a very high consumption of sulphuric acid, because the organic acids liberated from the organic salts are consumed by the yeast whereby the *p*H value is continually displaced to the alkaline side if sulphuric acid is not constantly added.

According to the present invention, it has been established, surprisingly, that not merely distillers' spent wash, but molasses spent wash of all types, including the effluent from the manufacture of glutamic acid, can be subjected successfully to the action of schizomycetes, actinomycetes or hyphomycetes or algae. In appropriate cases the spent wash may first be sterilized. This process is to be regarded as all the more surprising, as it was not to be anticipated that molasses spent wash of all kinds would be adapted for the culture of such fungi since, of the various micro-organisms, the yeasts occupy a special position and display peculiar anabolic processes. An element of surprise also derives from the fact that molasses spent wash, as effluent from the fermentation industries, contains only little assimilable nitrogen, as a large part of this has already been taken up by the microbes used in the fermentation and to a large extent only difficultly assimilable betaine nitrogen remains in the spent wash; nevertheless, the residual nitrogen compounds of the molasses spent wash are to a large extent assimilated by the schizomycetes, actinomycetes, hyphomycetes or algae.

In carrying out the process of this invention it is desirable to add to the spent wash an autolysate, plasmolysate, thermolysate, cytolysate or hydrolysate of micro-organisms propagated in the fermentation of molasses. In this way fermentation inhibitors released from the substrate by poisons or irritants are diminished or eliminated. In addition the growth and physiological activity of the schizomycetes, actinomycetes and hyphomycetes are enhanced in consequence of the content of high molecular nitrogen compounds and active principles of the added material. Moreover there is afforded the advantage that not only the spent wash but

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Price 2/-

also the cultures from molasses fermentation, otherwise unusable, as for example in the case of the manufacture of citric acid can be made of value.

Further in carrying out the process of this invention, nutrient salts and/or trace elements can be added to the spent wash in known manner.

It is advantageous to ferment the spent wash, for example that from the citric acid fermentation, with a vitamin B_{12}-forming micro-organism, such as streptomyces olivaceous in order to obtain from the spent wash an important nutritional factor with/without APF-activity. Similarly other micro-organisms can be substituted for the same purpose, for example those, such as streptomyces aureofaciens, which form antibiotics.

It is in general an advantage of the process of this invention that the addition of sulphuric acid in the fermentation of the spent wash is no longer necessary, and the fermentation is simpler and more economic.

The applicability of the invention is not restricted to the spent washes, hereinbefore mentioned by way of example, from the foodstuffs industries. Spent wash from the anaerobic methane fermentation can also be used. It is known that, in the methane fermentation, the organic material of the molasses is degraded to about 50% extent, without incurring loss of nitrogen. Thus, the spent wash is indicated as an especially appropriate nutrient medium for micro-organisms in view of its content of nitrogen and trace elements.

The decomposition of the micro-organisms, which in accordance with one method of carrying out the invention are also employed can, as already mentioned, be effected in various ways. This depends on the decomposability of the micro-organisms. In particularly simple cases sterilisation is sufficient to bring about decomposition. In other cases, autolysis or plasmolysis, known in themselves, can be used instead of thermolysis. In more difficult cases, for example with aspergillus types, it is desirable to make use of hydrolysis. The distinction here resides in the fact that in the case of autolysis the micro-organism's cell walls substantially remain and only the cell contents are obtained by the enzymatic process whilst in the case of hydrolysis the cell walls also are degraded and thereby pass into the nutrient composition. It has been established that for several of these micro-organisms the cell wall fission products are especially favourable nutrients. For example, Lactobacter bifidus grows on a yeast hydrolysate much better than on an autolysate.

The hydrolysis can be carried out partially or completely, in an acid or alkaline medium. The acid or lye concentration can vary widely, higher temperature being chosen for lower reagent concentrations. Generally the hydrolysis, which may be effected in an open or closed vessel, is not carried through to complete fission of the materials of the cell walls and cell contents. One will hydrolyse, for example, fungi from the citric acid, gluconic acid and itaconic acid fermentations, and from the manufacture of penicillin.

In special cases, for example with difficulty decomposable micro-organisms, it is also possible to achieve decomposition of fungi (cytolysis) by the action of particular other organisms, the mass being stirred if desired and in appropriate cases aerated.

For a more particular description of this process an example of cytolysis may be taken.

In a fermentation vessel, 50 kg. of moist fungus having a dry weight of about 11 kg. are inoculated with 5.1 of a suspension of Torula yeast obtained in submerged culture, and 25.1 of water are added. After 24 hours of relation of the vessel at 30—35° C. the by now viscous decomposed fungal mass is removed from the vessel. Through this decomposition about 40% of the dry substance of the mycelium is converted into a water-soluble form.

Instead of Torula yeast one can, for example, use streptomyces olivaceous or penicillium clariforme.

The invention will be described further by the following examples, without being restricted thereto.

EXAMPLE 1.

200 l. of molasses spent wash from the manufacture of alcohol, yeast or citric acid are treated with the usual quantity of calcium carbonate, cobalt chloride and where necessary carbohydrates, sterilized, brought to *p*H 5 and fermented with streptomyces olivaceous at 35° C. for 3—4 days for the production of a vitamin B_{12}-containing fodder. The final fermented mash, with a solids content of about 5% of which about half is protein, contains about 1 mgm. of vitamin B_{12} per litre.

EXAMPLE 2.

1000 kg. of pressed fungal substance from the manufacture of citric acid, thermolysed, 500 kg. of molasses spent wash, 80 l. of molasses, together with nutrient salts, are sterilized and fermented as in Example 1. The fungus can alternatively be hydrolysed or in particular cases autolysed or plasmolysed.

EXAMPLE 3.

For the cultivation of bacillus megatherium, the compositions for the nutrient medium given in Example 1 or 2 can be used, except that the molasses addition is increased by 10—20%.

EXAMPLE 4.

For the utilisation of the spent wash from the methane fermentation this is sterilized and used as in the foregoing examples, in like or greater amount, as nutrient substrate and fermentation-promoting additive. The sediment formed in the methane fermentation can be added to the effluent after autolysis or thermolysis.

EXAMPLE 5.

50 kg. of fresh, still moist mycelium from the citric acid fermentation are treated with 50 l. of mother liquor (spent wash) from the citric acid fermentation of molasses, mechanically comminuted, and sterilized. This mycelial broth is then put into a fermentation drum and inoculated with 10 l. of a shake culture of streptomyces olivaceous. After running for 2—4 days, during which time the temperature is kept at 25—30° C., the process is finished. The decomposed fungal mass is freed from water on drying rolls and finally ground. One obtains about 13 kg. of a brownish product which, by virtue of its content is growth-promoting active principles (vitamins, enzymes, etc.) is a notable fodder.

EXAMPLE 6.

20 kg. of pressed fungal mass from the submerged gluconic acid fermentation are mixed with 30 l. of molasses spent wash (distillers' spent wash) in a closed vessel fitted with a built-in, slow-speed paddle stirrer and sterilized. The cooled, viscous fungal mass is then inoculated with 5 l. of a suspension of streptomyces griseous developed in submerged culture, and held at 28—30° C. for 24—48 hours with constant stirring and weak aeration. The decomposed fungal mass is then emptied out and dried. There is obtained 7 kg. of dried material with an average moisture content of 5%, which can be used as fodder worked up for the isolation of its valuable constituents.

EXAMPLE 7.

4 kg. of washed, comminuted fungal carpet (Aspergillus wentii) from the non-submerged fermentative production of citric acid are treated with 20 l. of molasses spent wash from citric acid manufacture as obtained after precipitation of the calcium citrate (dry content about 10%) and 80 mgm. of cobalt nitrate, made up to 40 l. with water and sterilized. The *p*H value is brought to 6—7 with caustic soda. The cooled medium is now inoculated with 4 l. of a submerged culture of streptomyces olivaceous. The development of the inoculant is effected in a shake culture, for which a mycelium-molasses spent wash medium is very suitable. The inoculated culture medium is then aerated, with stirring, in a fermenter with 600 l/hour at 30° C. After 3—4 days the viscous medium is run out and carefully dried. One obtains about 2.5 kg. of a dry product with a vitamin B_{12} content of 10—30 micrograms per gram, which can be used as growth promoting fodder additive or worked up for the recovery of its constituents.

EXAMPLE 8.

200 l. of the molasses spent wash from glutamic acid manufacture are treated as set forth in Example 1; the finished mash, having a dry content of about 4% of which about half is protein, contains about 0.8 mgm. of vitamin B_{12} per litre.

EXAMPLE 9.

If vitamin B_{12} and/or antibiotics are to be recovered in a pure state from the products obtained in accordance with the foregoing examples, the microbes should be separated by centrifuging after a fermentation time of about 60 hours and the vitamins or antibiotics recovered. This can be done, for example, by solvent extraction.

The invention is not limited to the use of the three micro-organisms exemplified above, namely S. olivaceous, S. aureofaciens, and B. megatherium. Other micro-organisms which may be used include, for example, S. griseus, Penicillium claviforme, Lactobacillus caseii and Propionibacterium freudenreichii.

WHAT WE CLAIM IS:—

1. A process for the utilization of molasses spent wash as hereinbefore refined by treatment thereof, after sterilization where appropriate, with schizomycetes, actinomycetes, hyphomycetes or algae, to form a product suitable for use as an animal feeding stuff or nutritional factor.

2. A process as set forth in Claim 1 in which autolysates, plasmolysates, thermolysates, cytolysates or hydrolysates of micro-organisms propagated or employed in the production of the spent wash are added to the spent wash.

3. A process as set forth in any of the preceding claims, in which nutrient salts and/or trace elements are incorporated in the spent wash to promote the treatment thereof.

4. A process as set forth in Claim 2, in which the spent wash is derived from citric acid production and said micro-organisms are represented by the mycelium from such production, the treatment being effected with a vitamin B_{12}-forming micro-organism, suitably streptomyces olivaceous.

5. A process as set forth in Claim 4, modified in that the treatment is effected

with an antibiotic-forming organism, suitably streptomyces aureofaciens.

6. A process as set forth in Claim 2 in which the spent wash is from the methane fermentation.

7. A process as set forth in any of Claims 1—3 in which the spent wash is from glutamic acid production.

8. The utilization of molasses spent wash substantially as described in any of the foregoing examples.

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